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Short communication

IMPROVEMENT OF CALLOGENESIS AND SOMATIC EMBRYOGENESIS BY SELECTING OPTIMAL HORMONAL BALANCE IN SARNAV AND DESIREE POTATO VARIETIES

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Callus formation and somatic embryogenesis in potato varieties are effective ways to obtain an entire plant from a single cell. The implementation of embryogenesis is widely used in improving plant materials and creating new biotechnological varieties. In our research, the optimum level of 1-naphthaleneacetic acid (NAA) and 6-benzyl aminopurine (BAP) was determined in the formation of callus tissue in the local Sarnav potato variety, and the Desiree variety taken as a control. Leaf and stem explants were used in the processes of callus tissue formation and somatic embryogenesis of both varieties. We tried various plant growth regulators for callus formation and somatic embryogenesis in different ratios. The 1 mg/L NAA and 1.5 mg/L BAP were established as the best option for callus formation resulting in 92 and 100% development of callus in internodes of Desiree and Sarnav varieties, respectively. The 0.1 mg/L BAP and 0.1 mg/L gibberellic acid (GA_3) resulted in almost 80% development of somatic embryogenesis in both varieties and were found as the most optimum option. These results showed that regenerated plants can be obtained from the Sarnav potato variety by somatic embryogenesis. This method can be applied to the Sarnav variety for genetic transformation studies.

Key words: *Solanum tuberosum*, callus, somatic embryogenesis, 1-naphthaleneacetic acid, 6-benzyl amino purine, gibberellic acid

One of the significant problems in potato (*Solanum tuberosum* L.) cultivation is its susceptibility to viruses, bacteria, and various pests. Here, obtaining genetically enriched plants and regenerates from callus tissues through somatic embryogenesis is important for preserving genetic materials from generation to generation (Miguel & Marum 2011).

Different ratios of BAP and NAA were used for *in vitro* microclonal propagation of Gudiene and Belete potato varieties. In the Gudiene variety, 1.5 mg/L BAP + 3.0 mg/L NAA revealed higher results in shoot development, while 1.0 mg/L BAP + 2.0 mg/L NAA had the maximum effect in the Belete variety (Hajare *et al*. 2021). Leyser (2001) reviews how

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cells can take up and distribute auxin during the development of leaves, roots, and stems in plants, and how it has different effects on tissues. The results of *in vitro* regeneration of plant tissues and somatic embryos demonstrated the interaction of auxin and cytokinin in the nutrient medium is regulated by different mechanisms (Cheng *et al*. 2013).

In potatoes, somatic embryo development was induced by phytohormones cytokinin and auxins, such as naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and benzyladenine (BA) (Jayasree *et al*. 2001). Regenerated plants, obtained through somatic embryos, maintain stability in transmitting genetic material from generation to generation. In this study, we aimed to determine the optimal concentration of benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) for callus formation, as well as BAP and gibberellic acid (GA_3) for somatic embryogenesis in the local Sarnav potato variety. We compared the results of the local variety with those of the Desiree variety, which is often used as the control.

The research was conducted at the Transgenomics and Tissue Culture Laboratory of the Centre of Genomics and Bioinformatics of the Academy of Sciences of Uzbekistan, focusing on the *in vitro* cultivation of Sarnav and Desiree potato varieties. In this work, 100 samples of internodal stem and leaf explants were used. Explants were cultivated at 20 – 22°C, a humidity of 72 – 74%, and a photoperiod of 16 h of light (2,000 – 3,000 lux)/8 h of dark. For preparing 1 L of callus induction medium, 4.31 g of MS mixture (Murashige & Skoog 1962), 30 g of sucrose, and 8 g of phyto-agar were used. Different mass ratios of NAA and BAP were used to establish the optimum ratio: 1.5: 1 mg/L for the M1 culture medium; 1: 1.5 mg/L for the M2 medium; 2: 2 mg/L for the M3. Only NAA was included for the M4 (1.5 mg/L), M5 (1 mg/L); and M6 (3 mg/L) culture media. For the preparation of 1 L of somatic embryogenesis nutrient medium (SE), 4.31 g MS powder (Murashige & Skoog 1962), 20 g sucrose, 0.1 g myo-inositol, 8 g phyto-agar, and various ratios of BAP and GA_3 (1: 0.5 mg/L for SE1; 0.4: 0.1 mg/L for SE2; 0.5: 0.2 mg/L for SE3; 0.1: 0.1 mg/L for SE4) were used.

Callus tissue formation was observed after 5 – 7 days in all nutrient media on the cut surface of the explants. After subculturing in a nutrient medium for two months, the frequency of callus formation was evaluated. Statistical analyses were carried out by F-statistic value criteria. Callus induction frequency was calculated using the formula below (Harun-Or-Rashid *et al*. 2001).

$$\text{Callus induction frequency } \% = \frac{N_{\text{explants produced calli}}}{N_{\text{explants cultured}}} \times 100$$

The highest callus formation was achieved in Sarnav leaf explants grown in M2, M3, M4, and M5 culture media. In M4, M5, and M6 media, in which NAA alone was used, callus formation rates ranged from 65 to 100% in leaf explants of Sarnav and Desiree varieties. The highest frequencies of callus formation in both varieties were achieved in the M2 nutrient medium containing 1 mg/l NAA and 1.5 mg/L BAP (Figure 1). Our findings align with those obtained by Harun-Or-Rashid *et al*. (2001), who also observed similar callus formation rates. Callus formation on internodal explants was lower compared to leaf explants in both varieties. In the M2 nutrient medium, the frequency of the callus formation from internodal explants was the highest and reached 92% in the Desiree and 100% in the Sarnav explants.

In the callus formation, compact pale green calli were initially formed on both varieties' internodal and leaf explants. During their development process, the colour of the callus tissues changed and differed depending on the explant type. Light green callus tissue was formed on the leaf explants, and a yellow white callus was formed on the stem explants (Figure 2). From the 8th week, it changed to light green, green, and greenish yellow colours. Greening occurred due to the development of chloroplasts in the callus cells under the influence of light. The colour and structure of the callus tissue ensure the continuity of the cell proliferation process. This increases the probability of obtaining somatic embryos from these cells.

Somatic embryos appeared mainly in light green, yellow and green tissues, and increased the chance of plant regeneration. This can be explained by the fact that the browning of callus tissue is associated with the excess amount of phenols in tissues inhibiting plant growth (Kumar *et al*. 2015). In the leaf explants of the Sarnav variety, a compact pale green

callus appeared in the M3 nutrient medium, which turned brown after 2 months. Probably, due to the high content of auxin and cytokinins, the process of cell division was accelerated and led to the ageing process.

The optimum ratios of various plant growth regulators vary in different plants and even varieties. When several ratios of BAP and NAA were used for the Cardinal potato variety, the 2.0 mg/L BAP and

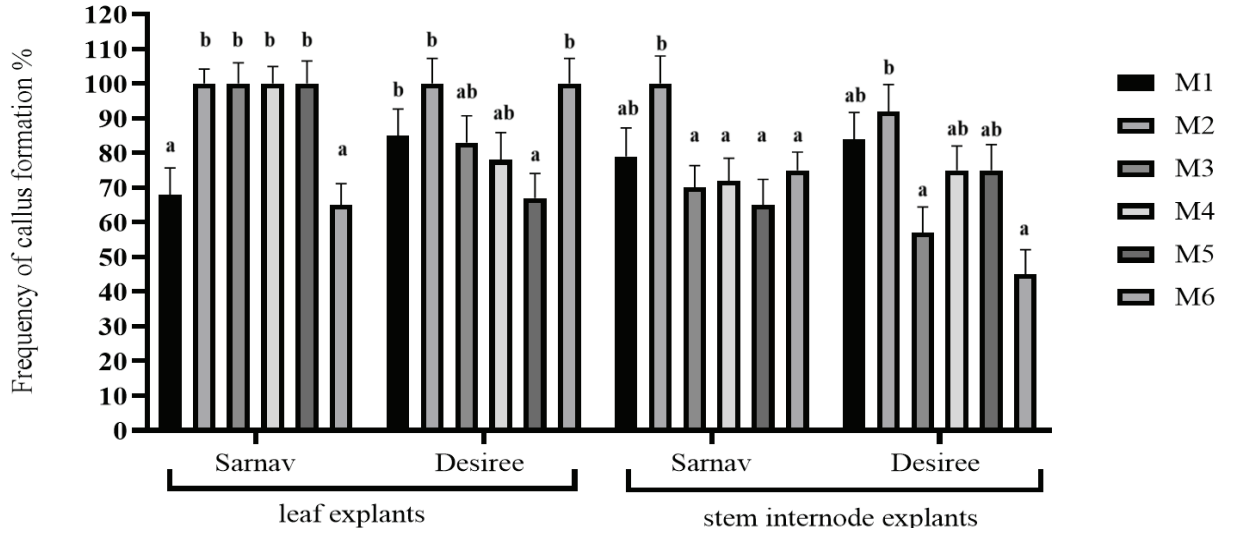

Figure 1. Effect of different concentrations of NAA/BAP and NAA on callus proliferation of leaf and stem internode explants of Sarnav and Desiree varieties. a, b – significant difference $P \leq 0.01$, respectively.
Note: NAA – 1-naphthaleneacetic acid; BAP – 6-benzyl aminopurine; M1-M6 – MS (Murashige & Skoog 1962) culture media enriched with different ratios of plant growth regulators: M1 (1.5 mg/L NAA: 1 mg/L BAP), M2 (1 mg/L NAA: 1.5 mg/L BAP), M3 (2 mg/L NAA: 2 mg/L BAP), M4 (1.5 mg/L NAA), M5 (1 mg/L NAA), M6 (3 mg/L NAA).

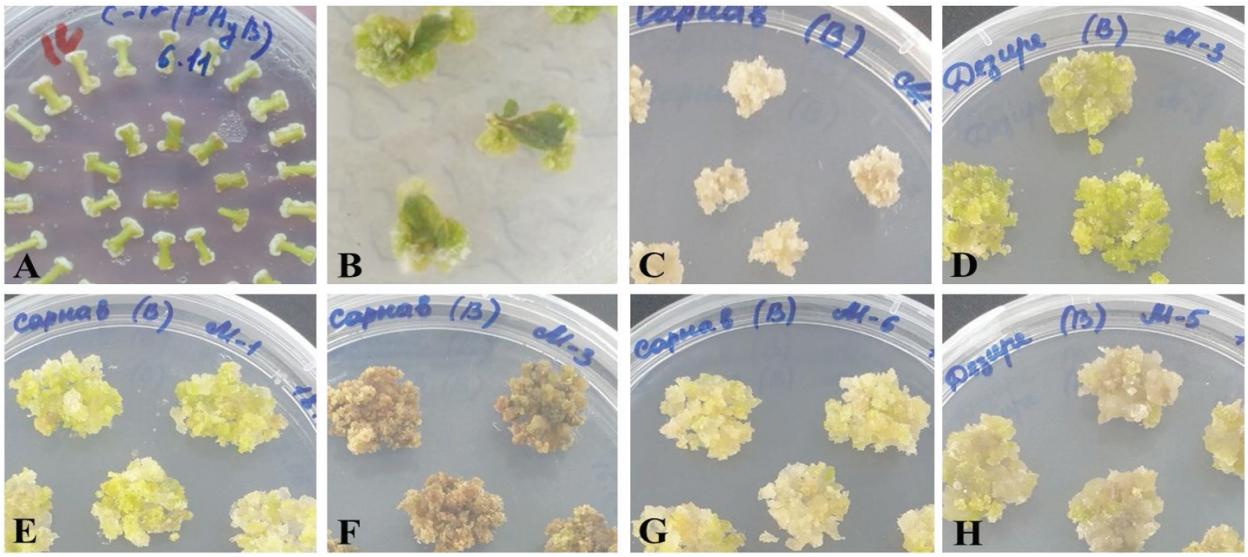

Figure 2 (A – H). Effect of auxin and cytokinin concentration on callus proliferation in potato explants (internode stems and leaves) grown *in vitro*.
Note: A – Yellowish white callus formed from internode stem explant in M2 nutrient medium of Sarnav variety; B – Yellowish white callus formed from leaf explant in M2 (1 mg/L NAA and 1.5 mg/L BAP) nutrient medium of Sarnav variety; C – Yellowish white callus after 20 days in M1 (1.5 mg/L NAA and 1 mg/L BAP) nutrient medium of Sarnav variety; D – Green callus obtained from leaf explants of Desiree variety in M3 (2 mg/L of NAA and 2 mg/L BAP) nutrient medium; E – Light green callus obtained from Sarnav variety in M1 (1.5 mg/L of NAA and 1 mg/L BAP) nutrient medium; F – Brown callus obtained from Sarnav variety in M3 (2 mg/L NAA and 2 mg/L BAP) nutrient medium; G – Green yellow callus of variety in M6 (NAA 2 mg/L) nutrient medium; H – Brownish green callus of Desiree variety in M5 (NAA 1 mg/L) nutrient medium. M1-M6; NAA; BAP – see Figure 1.

2.5 mg/L NAA caused 95% callus formation and 80% regeneration efficiency (Yasmin *et al*. 2003).

Auxins and cytokinins are essential for forming callus tissue and somatic embryogenesis in plants (Przybył *et al*. 2020). When these chemicals are not sufficient, cell division slows down. Therefore, there is a need to use them exogenously. In our study, the M2 nutrient medium containing 1.0 mg/L NAA and 1.5 mg/L BAP resulted in higher callus formation in Sarnav and Desiree varieties. This concentration was found to be effective for both varieties. Later, the callus tissues obtained from leaf and internodal stem explants were transferred to the MS nutrient medium prepared for somatic embryogenesis (SE1 to SE4). In different plants, various concentrations of plant growth regulators have been found to yield optimal results (Nassar *et al*. 2015). In this study, we used MS nutrient media (SE1, SE2, SE3, and SE4) enriched with different ratios of BAP and GA_3 (1 : 0.5; 0.4 : 0.1; 0.5 : 0.2; 0.1 : 0.1 mg/L). Embryogenic cells appeared two weeks after transfer to a new nutrient medium.

Embryogenic cells were distinguished from non-embryogenic cells by their rounded shape, and when we observed the embryogenic cells under the microscope, different stages were detected (Figure 3 (A–C)). The onset of somatic embryogenesis in callus tissues depended on the composition of the nutrient medium. In this process, the cells differentiate and form a spherical structure. Somatic embryos initially developed in the nutrient medium containing 0.1 mg/L BAP and 0.1 mg/L of GA_3.

In this work, we observed the embryos of the globular, heart, and torpedo stages in the explants of potato leaves and internode stems. Several works have been carried out on obtaining regenerates by somatic embryogenesis in potatoes. Globular-, heart-, and torpedo-shaped embryos were obtained when 2,4-D was used (Sharma & Millam 2004). When 0.5 mg/L 2,4-D was applied to the internodal stems of potato, callus tissue was obtained and embryogenic cells appeared (Vargas *et al.* 2005). Khatun *et al*. (2003) achieved 90% callus formation by using 2.5 mg/L 2,4-D. Nassar *et al.* (2015) summarised that the doses 3 – 8 mg/L of NAA with 0.25 – 0.5 mg/L of BAP were successfully used to obtain callus from potato explants, and the doses 0.4 – 1 mg/L of BAP with the 0.1 – 0.4 mg/L of GA_3 were efficient to obtain somatic embryos.

The total percentage of embryos and the number of globular-, heart-, and torpedo-shaped stages were evaluated at the 10th week of their development. The percentage of embryo formation was calculated based on the number of explants that made 100 samples in leaves and inter-joint stem explants placed for callus formation in nutrient media (SE1, SE2, SE3, and SE4). Accordingly, were calculated the number of embryos in each nutrient medium. The total number of embryos in the Sarnav variety leaf explants made 50 (50%) in SE1 medium; 57, 49, and 80% results were observed in SE2, SE3 and SE4 culture media, respectively (Figure 4). In internodal stem explants, the total number of embryos made 48, 55, 45, and 70 in SE1, SE2, SE3, and SE4 culture media, respectively (Figure 5). Similar patterns were observed between the development of somat-

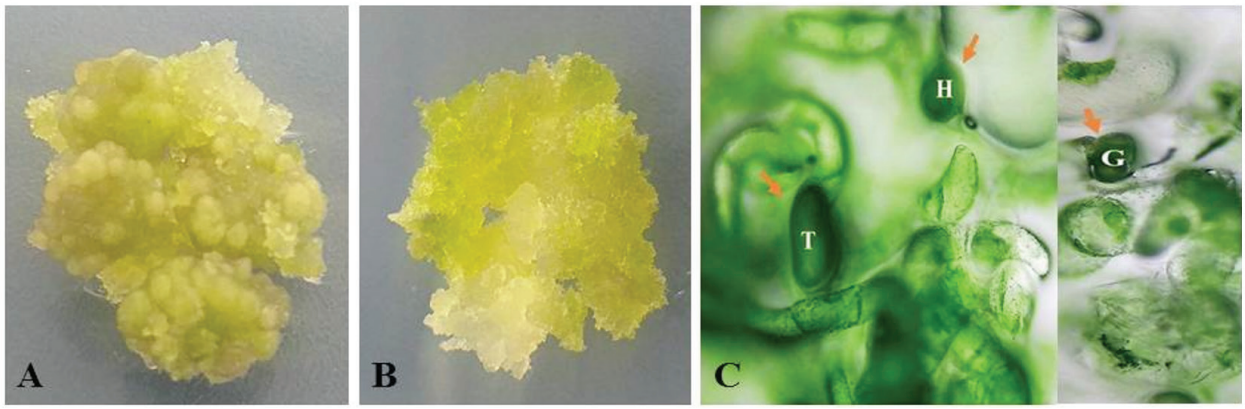

Figure 3 (A – C). Sarnav potato callus tissue in a SE4 somatic embryogenesis nutrient medium holding 0.1 mg/L of BAP and GA_3 each. A – embryogenic callus tissue; B – non-embryogenic callus tissue; C – Stages of development of the somatic embryo: G – globular stage embryo; H – heart stage embryo; T – torpedo stage embryo.
Note: BAP – 6-benzyl aminopurine; GA_3 – gibberellic acid.

ic embryos obtained from leaf and internodal stem explants. The SE4 culture medium that contain 0.1 mg/L BAP and 0.1 mg/L GA_3 resulted in the highest level of embryo formation in globular- and heart-shaped stages on both leaf (Figure 4) and internodal stem explants (Figure 5).

Direct somatic embryogenesis from leaf and internodal stem explants of the 'Kufri Chipsona 2' potato variety was induced by 2.5 µM 2,4-D and 1.0 mM BA. In their study, the embryos changed from heart-shaped to torpedo-shaped; after 8 weeks, they became cotyledon-shaped (Kaur *et al.* 2018). In another work, somatic embryos were formed in the explants of potato internodal stem, leaf, microtuber, and root. At first, light green calli appeared from the cut place of the inter-articular stem. These results were obtained in nutrient media containing auxin and cytokinins: 19 µM IAA, 0.15 µM TDZ, 0.15 µM BAP, 12 µM zeatin, and 0.55 µM GA_3 (Seabrook & Douglass 2001). Sharma *et al.* (2007) showed that auxin is of primary importance in the process of somatic embryogenesis in potato plants. Positive results were achieved when 20 µM 2,4-D was applied to internodal stem explants of potato. The efficiency of BAP together with other plant growth regulators on the somatic embryogenesis, indicated in above men-

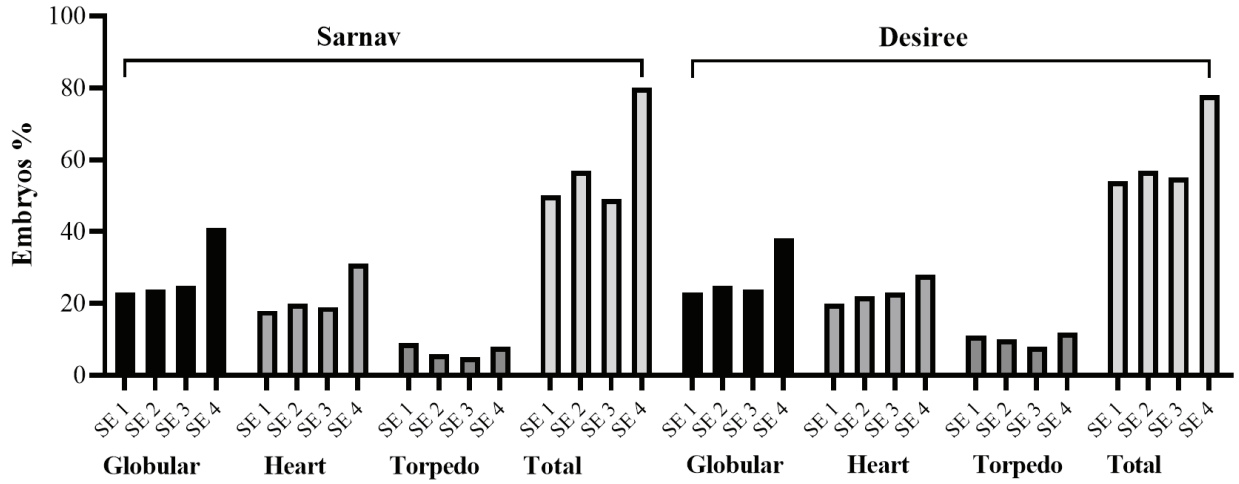

Figure 4. Percentage of somatic embryos with a given developmental stage of embryos obtained from leaf explants in media SE1 (1: 0.5 mg/L BAP and GA_3); SE2 (0.4: 0.1 mg/L BAP and GA_3); SE3 (0.5: 0.2 mg/L BAP and GA_3); SE4 (0.1: 0.1 mg/L BAP and GA_3).
Note: SE1 – SE4 – Somatic embryogenesis culture media enriched with plant growth regulators; BAP, GA_3 – see Figure 3.

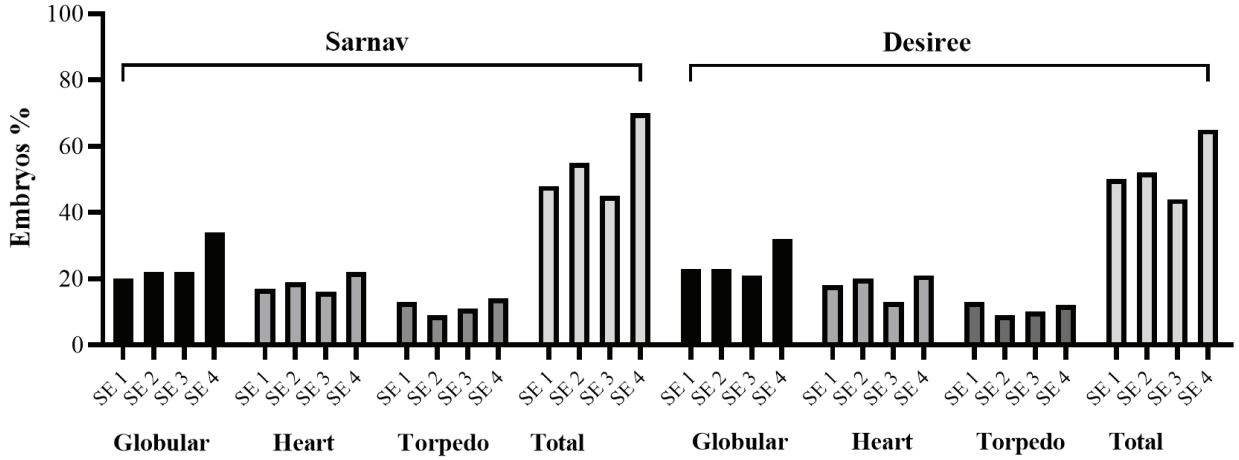

Figure 5. Percentage of somatic embryos with a given developmental stage of embryos obtained from internodal stem explants in media SE1 (1: 0.5 mg/L BAP and GA_3); SE2 (0.4: 0.1 mg/L BAP and GA_3); SE3 (0.5: 0.2 mg/L BAP and GA_3); SE4 (0.1: 0.1 mg/L BAP and GA_3).
Note: SE1 – SE4 – Somatic embryogenesis culture media enriched with plant growth regulators; BAP, GA_3 – see Figure 3.

tioned works, corresponds with our results. The formation of somatic embryogenesis in various stages, observed in our work, is in correspondence with other results (Seabrook & Douglass 2001; Sharma *et al.* 2007; Kaur *et al.* 2018).

CONCLUSIONS

In this study, callus induction and somatic embryogenesis were successfully achieved in both Sarnav and Desiree potato varieties using BAP-, NAA-, and GA_3-enriched variants of MS medium. The highest efficiency for callus formation was observed at the 1: 1.5 mg/L ratio of NAA and BAP, resulting in 92% efficiency for the Desiree variety and 100% efficiency for the Sarnav variety. The Sarnav variety exhibited greater callus formation in comparison to the Desiree variety in other nutrient media. Furthermore, somatic embryo formation was highly efficient in internode stem explants using BAP and GA_3 at the 0.1: 0.1 mg/L ratio, with both varieties achieving a 65 – 70% success rate. The Sarnav variety demonstrated 80% efficiency in leaf explants, while the Desiree variety showed 78%. These results highlight the suitability of the local Sarnav variety for somatic embryogenesis and provide valuable insights for future genetic transformation research. These results will be used for genetic transformation research in the future.

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Conflict of interests: The authors declare that they have no conflict of interest.

REFERENCES

Cheng, Z.J., Wang, L., Sun, W., Zhang, Y., Zhou, C., Su, Y.H., Li, W., Sun, T.T., Zhao, X.Y., Li, X.G., Cheng, Y., Zhao, Y., Xie, Q., and Zhang, X.S. (2013). Pattern of auxin and cytokinin responses for shoot meristem induction results from regulation of cytokinin biosynthesis by auxin response factor$_3$. *Plant Physiology, 161*(1), 240 – 251. DOI:10.1104/pp.112.203166.

Hajare, S.T., Chauhan, N.M. and Kassa, G. (2021). Effect of growth regulators on *in vitro* micropropagation of potato (*Solanum tuberosum* L.) Gudiene and Belete varieties from Ethiopia. *Scientific World Journal*, 5928769. DOI: 10.1155/2021/5928769.

Harun-Or-Rashid, M., Shahinul Islam, S.M., Bari Miah, M.A., and Subramaniam, S. (2001). *In vitro* screening of calli and evaluation their physiological states for the enhancement of regeneration efficiency in various potato (*Solanum tuberosum* L.) genotypes. *Biocatalysis and Agricultural Biotechnology*, *28*, 101715. DOI:10.1016/j.bcab.2020.101715.

Jayasree, T., Pavan, U., Ramesh, M., Rao A.V., Reddy, J.M., and Sadanandam, A. (2001). Somatic embryogenesis from leaf culture of potato. *Plant Cell, Tissue and Organ Culture*, *64*, 13 – 17. DOI:10.1023/A:1010697608689.

Kaur, A., Reddy, M.S. and Kumar, A. (2018). Direct somatic embryogenesis of potato [*Solanum tuberosum* (L.)] cultivar 'Kufri Chipsona 2'. *Plant Cell, Tissue and Organ Culture*, *134*, 457 – 466. DOI:10.1007/s11240-018-1435-4.

Khatun, N., Bari, M.A., Islam, R., Huda, S., Siddique, N.A., Rahman, M.H., and Mollah, M.U. (2003). Callus induction and regeneration from nodal segment of potato cultivar Diamant. *Journal of Biological Sciences*, *3*, 1101 – 1106. DOI:10.3923/jbs.2003.1101.1106.

Kumar, G.P., Subiramani, S., Govindarajan, S., Sadasivam, V., Manickam, V., Mogilicherla, K., Thiruppathi, S.K., and Narayanasamy, J. (2015). Evaluation of different carbon sources for high frequency callus culture with reduced phenolic secretion in cotton (*Gossypium hirsutum* L.) cv. SVPR-2. *Biotechnology Reports*, *7,* 72 – 80. DOI:10.1016/j.btre.2015.05.005.

Leyser, O. (2001). Auxin signaling: The beginning, the middle and the end. *Current Opinion in Plant Biology*, *4*(5), 382 – 386. DOI:10.1016/S1369-5266(00)00189-8.

Liang, H., Xiong, Y., Guo, B., Yan, H., Jian, Sh., Ren, H., Zhang, X., Li, Y., Zeng, S., Wu, K., Zheng, F., Silva, J.T., Xiong, Y., and Ma, G. (2020). Shoot organogenesis and somatic embryogenesis from leaf and root explants of *Scaevola sericea*. *Scientific Reports*, *10,* 11343. DOI:10.1038/s41598-020-68084-1.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473 – 497. DOI:10.1111/j.1399-3054.1962.tb08052.x.

Miguel, C. and Marum, L. (2011). An epigenetic view of plant cells cultured *in vitro*: somaclonal variation and beyond. *Journal of Experimental Botany*, *62*(11) 3713 – 3725. DOI:10.1093/jxb/err155.

Nassar, A.M.K., Kubow, S. and Donnelly, D.J. (2015). Somatic embryogenesis for potato (*Solanum tuberosum* L.) improvement. In Li, X-Q, Donnelly, D.J. and Jensen T. G. (Eds.) *Somatic genome manipulation: Advances, methods, and applications.* New York: Springer. pp. 169 – 198. DOI:10.1007/978-1-4939-2389-2_8.

Przybył, T.H., Ratajczak, E., Obarska, A., and Pers-Kamczyc, E. (2020). Different roles of auxins in somatic embryogenesis efficiency in two Picea species. *International Journal of Molecular Sciences, 21*(9), 3394. DOI:10.3390%2Fijms21093394.

Seabrook, J.E.A. and Douglass, L.K. (2001). Somatic embryogenesis on various potato tissues from a range of genotypes and ploidy levels. *Plant Cell Reports, 20*, 175 – 182. DOI: 10.1007/s002990000305.

Sharma, S.K., Bryan, G.J. and Millam, S. (2007). Auxin pulse treatment holds the potential to enhance efficiency and practicability of somatic embryogenesis in potato. *Plant Cell Reports, 26*, 945 – 950. DOI:10.1007/s00299-007-0319-6.

Sharma, S. K. and Millam, S. (2004). Somatic embryogenesis in *Solanum tuberosum* L.: a histological examination of key developmental stages. *Plant Cell Reports, 23,* 115 – 119. DOI:10.1007/s00299-004-0814-y.

Vargas, T. E., Garcı´a, D. E. and Oropeza, M. (2005). Somatic embryogenesis in *Solanum tuberosum* from cell suspension cultures: histological analysis and extracellular protein patterns. *Journal of Plant Physiology, 162*, 449 – 456. DOI:10.1016/j.jplph.2004.07.001.

Yasmin, S., Nasiruddin, K. M., Begum, R., and Talukder, S. K. (2003). Regeneration and establishment of potato plantlets through callus formation with BAP and NAA. *Asian Journal of Plant Sciences*, *2*(12), 936 – 940. DOI:10.3923/ajps.2003.936.940.

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